REMARKS

Amendment to the claims

New claim 29 has been introduced to specify that the monascus ruber is limited to strain F125 or monascus ruber strain F125 M1-4 as disclosed in the specification on page 17, lines 11-28.

Claim Rejections – 35 USC § 103

Claims 11-28 were rejected under 35 USC §103(a) as being unpatentable over Mezaache et al (US 6,165,512) in view of Kelly (US 5,830,887), Giese et al (5,674,892) and Merck Index 11 ed. S. Budavari, editor, P686, pp878-879, 1989. Applicants traverse this rejection.

Mezaache et al disclose compositions useful for making taste-masked oral dosage forms. The preferred dosage form is tablets (abstract). Lozenges are also mentioned. The compositions utilize "liquiflash particles". Mezaache et al disclose at column 8, lines 7-9 that "The liquiflash particles used herein contain one or more active ingredients, such as bio-affecting ingredients, such as bio-affecting agents. These are typically prescription or over the counter medications."

Mezaache further states at column 8, lines 10-11 that "the actives ingredients useful herein can be selected from a large group of therapeutic agents" and goes on to list alphabetically many classes of materials and specific examples. The compilation starts at column 8 line 12 and continues to column 10, line 12. Lovastatin is listed under the "L's" along with lidocaine and luteinizing hormones.

Mezaache is silent with respect to any food products, any extracts of fermented soy, and soy fermented with Monascus Ruber and extracted with either ethanol or an edible oil.

Kelly discloses a health supplement specifically enriched for isoflavones selected from genistein, daizzein, formononetin and biochanin A or their natural glycoside form or their analogs in sufficient amounts to improve the health of humans (column 5, lines 53-57). Kelly further teaches that clovers are the preferred sources (column 8, lines 1-3). According to Kelly, soybean flour may also be used for enrichment of phyto-oestrogens but the substantially poorer yields of isoflavones compared to clover (~10%) increases cost and generates waste.

Kelly is silent with respect to any specific food product, any extracts of fermented soy, and soy fermented with Monascus Ruber and extracted with either ethanol or an edible oil.

Giese et al disclose a method for selectively inhibiting a kinase which comprises contacting the kinase with a molecule of the general structure shown at column 3, lines 30-45. Genistein is not encompassed by this structure (compare attached structure of genistein with Giese et al patent). Giese et al make only one reference to genestein at column 8, line 46 "The PTK inhibitors quercetin, genistein, and stauorsporin inhibit many other tyronase inhibitors and as a result of their lack of specificity are highly cytotoxic". (emphasis added). Thus, the teaching is that genestein is a less preferred material than the inventive compounds for treatment in humans because of its poor selectivity and cytotoxicity. Applicants are puzzled by the Office's citation of Giese et al because the above teaching would suggest genistein would not be a good thing to add to foods.

Giese et al are silent with respect to any food product, any extracts of fermented soy, and soy fermented with Monascus Ruber and extracted with either ethanol or an edible oil.

Merck Index was cited by the Examiner for its teaching that Lovastatin has been isolated from Monascus Ruber citing A. Endo, J Antibiot. 32, 852 (1979). A copy of this article is attached. Endo states "M. Ruber was grown aerobically at 28° C in a culture containing 6% glucose, 2.5% peptone, 0.5% corn steep liquor and 0.5% ammonium chloride for 10 days". Endo goes on to describe the extraction and purification of Monacolin K (AKA lovastatin) which involves extraction of a pellet with ethyl acetate, treatment with benzene and extensive work-up with various solvents systems.

Thus, the Merck index citation (Endo article) is in fact silent regarding fermentation of a substrate comprising more than 50% soy ingredients with monascus rubber. The article is also silent about extracting any fermentation product with ethanol or an edible oil and adding the extract directly to foods.

In contrast, applicants invention is directed to specific food products which incorporate an ethanol or edible oil extract of a fermentation product formed by fermenting a substrate that is at least 50% soy ingredients with monascus rubber.

Applicants recognized the beneficial health effects of statins, polyphenols, saponins and phytosterols, etc. and the desirability of their incorporation in foods for health reasons (see introduction and page 4 lines 5-15). The problem they solved was to do this in an effective and practical way. Applicants discovered that an edible oil extract of the fermentation product of soy ingredients (e.g., crushed soybeans) fermented with monascus rubber in a certain way contained a collection of beneficial ingredients which they have described as "soy actives"(page 8, lines 15-17). Moreover, surprisingly, the extracts could be incorporated directly in the food product without extensive purification because, in contrast to prior art fermentations with monascus, their soy fermentation extracts were not highly colored and thus did not discolor the food products. Thus, applicants found a practical way to incorporate a collection of natural and beneficial ingredients in food without the expense of adding highly purified individual components.

Applicants' invention differs from the prior art in at least the following elements which are not disclosed either explicitly or implicitly in the collection of reference cited in the Office Action;

- "A food product selected from the group consisting of a margarine, a dressing, a sweet, a cereal bar, a breakfast cereal and a beverage". Applicants respectfully submit that oral dosage forms (tables and lozenges) that contain drugs not approved for use in foods (e.g., acetaminophen) and food supplements do not in fact constitute food products.

- The above selected food product comprises "an extract of a fermentation product formed by fermenting a substrate comprising more than 50% by weight of soy ingredients with a statins producing monascus ruber fungus wherein said soy ingredients are selected from the group consisting of whole soybeans, crushed whole soybeans, soy protein, soy milk and soy flakes". None of the references mention fermentation of a soy substrate.

- The above soy based fermentation product that "comprises one or more statins and one or more polyphenols". None of the references even hints that the fermentation of soy ingredients with monascus rubber bacteria provides a collection of beneficial "soy actives" which include statins and polyphenols, and none suggest that these soy actives could be simply extracted and the extract directly added to foods without extensive purification.

- The above soy based product that "has a Hue a* value less than 20". The Examiner dismissed this element stating that "synthetic lovastatin and genistein are well-known in the art in view of the Merck Index; it would be obvious to one of ordinary skill to use synthetic actives to maximize their purity". Applicants are not claiming synthetic actives. Applicants' are not adding synthetic actives to the soy fermentation extract. The soy actives come directly from the fermentation process (i.e., are part of

the fermentation product) as recited in the claim and construed by the specification (page 4, lines 17-21 and the Examples). Having low Hue a* value allows the extract (which contains a variety of soy actives and not just lovastatin and genistein) to have an acceptable color so that it can be directly added to the food. In fact, the Hue a* value of an extract such as the Endo extract, would be totally irrelevant when the target is a highly purified product since the numerous work up steps of extraction, recrystalization, etc. takes care of this issue, i.e., the purification work-up intrinsically removes color. It is only when a simple extract, e.g., a vegetable oil extract, is used that color becomes an issue.

- "wherein said extract is an ethanol extract or an edible oil extract". Endo (Merck Index citation) employed ethyl acetate and benzene extraction to extract lovastatin from the glucose/corn fermentation. These treatments would not be acceptable for a food ingredient.

Thus, setting aside any consideration of logic of combining these disparate "drug" references to a food application, even if all the cited references were combined, the combination would not have produced applicants' claimed invention because the combination does not contain any one of the above elements let alone all of them.

Absent a disclosure of the above cited key elements, the references do not present a *prima facie* case of obviousness.

In light of the above remarks, applicants respectfully request that the 103 rejection over Mezaache et al (US 6,165,512) in view of Kelly (US 5,830,887), Giese et al (5,674,892) and Merck Index 11 ed. S. Budavari, editor, P686, pp878-879, 1989 be reconsidered and withdrawn and that the application be allowed to issue without further delay.

If a telephone conversation would be of assistance in advancing prosecution of the subject application, applicants' undersigned agent invites the Examiner to telephone him at the number provided.

Respectfully submitted,

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CERHR Home >> CERHR Chemicals >> Genistein

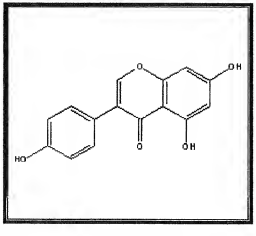
Chemicals - Genistein

Molecular Formula: $C_{15}H_{10}O_5$

Synonyms: 4',5,7-Trihydroxyisoflavone; Genistein

CAS Registry Number: 446-72-0

Chemical Structure:



Genistein Evaluation

- Meeting Summary
- Expert Panel Report
- Draft Expert Panel Report
- Draft Brief
- Public comments on Genistein Evaluation

Back to Top

Page last updated on 11/07/2006

MONACOLIN K, A NEW HYPOCHOLESTEROLEMIC AGENT PRODUCED BY A *MONASCUS* SPECIES

Sir:

In previous papers from our laboratory, ML-236B, a metabolite of *Penicillium citrinum* that was isolated as an inhibitor of cholesterol synthesis[1], was shown to have hypocholesterolemic activity in several animal species[2,3]. Further work in this laboratory to search for microbial metabolites having cholesterol-lowering activity led to the isolation of a new active compound (designated as monacolin K) produced by a *Monascus* species. The present paper describes isolation, physical and chemical properties and hypocholesterolemic effects of monacolin K.

The strain of *Monascus* employed in the production of monacolin K, which was isolated from a food sample collected in Thailand, was classified as *Monascus ruber* and designated as *Monascus ruber* No. 1005.

M. ruber No. 1005 was grown aerobically at 28°C in a medium containing 6% glucose, 2.5% peptone, 0.5% corn steep liquor (Corn Products Co., U.S.A.) and 0.5% ammonium chloride for 10 days. From the culture filtrate (5 liters), monacolin K was extracted with 5 liters of ethyl acetate at pH 3 and the extract was concentrated *in vacuo* to dryness. The resultant pellet was dissolved in 100 ml of benzene and the insoluble materials were removed by filtration. The filtrate was washed twice with 100 ml of 5% $NaCO_3$ and then mixed with 100 ml of 0.2 N NaOH with stirring at room temperature for 2 hours. The aqueous layers were pooled, adjusted to pH 3 with 6 N HCl and extracted twice with 100 ml of ethyl acetate. The solvent layer was collected and evaporated to dryness, giving 260 mg of an oily substance. This material was dissolved in a small volume of benzene, from which monacolin K was obtained as crystals. The compound was recrystallized from aqueous acetone, giving 87 mg of monacolin K as colorless crystals.

Monacolin K melted at 157~159°C (dec.) and had a $[\alpha]_D^{25}$ value of +307.6° (*c* 1, methanol). The molecular formula, $C_{24}H_{36}O_5$ (Mw 404), was obtained by elemental analysis (Calcd.: C 71.31, H 8.91, O 19.78%; Found C 71.56, H 8.85, O 19.59%) and high resolution mass spectroscopy. The UV spectrum (methanol) showed maxima at 229, 237 and 246 nm ($E^{1\%}_{1cm}$ 550, 650 and 430, respectively) (Fig. 1). The IR spectrum (KBr) showed absorption bands at 3550, 2970, 1696 and 1220 cm^{-1} (Fig. 2). The ^{13}C-NMR spectrum (CD_3OD) indicated the presence of 2 ester carbonyl carbons (δ 173.29 and 178.16), 4 methyl carbons (δ 12.18, 14.13, 16.62 and 23.39) and methylene and methine carbons (Fig. 3). In addition to peaks at (*m/e*) 404 (M^+), 302 (M−102), 284 (M−120) and 224 (M−180), prominent peaks in the mass spectrum of monacolin K were observed at 198 (M−206), 172 (M−232), 159 (M−245) and 157 (M−247) (Fig. 4). Monacolin K was soluble in methanol, ethanol, acetone, chloroform and benzene but not soluble in *n*-hexane and petroleum ether. The Rf value in TLC (Merck, Kieselgel $60F_{254}$) was 0.47 in dichloromethane - acetone (4: 1).

The LD_{50} of monacolin K in mice (oral administration) was over 1,000 mg/kg. Hypocholesterolemic activity of monacolin K was demonstrated in the two experiments described below. Male Wistar-Imamichi rats (240~280 g) were injected intraventrically with 400 mg/kg of the detergent Triton WR-1339 (Ruger Chemical Co., U.S.A.) and intraperitoneally with 10 mg/kg of monacolin K suspended in saline. Control animals received Triton and saline alone. After 14 hours, the animals were sacrificed and plasma

Fig. 1. UV spectrum of monacolin K (in methanol).

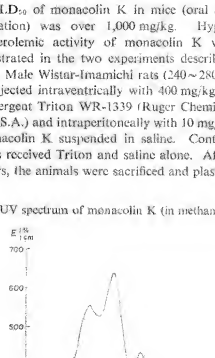


Fig. 2. IR spectrum of monacolin K (KBr).

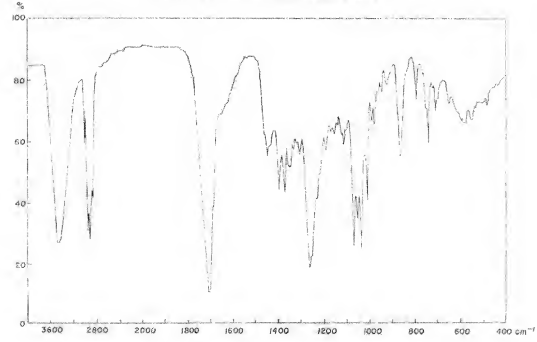
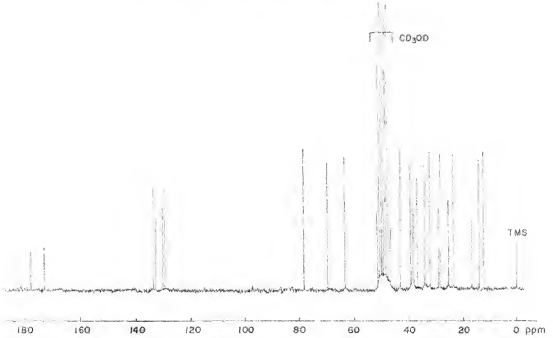


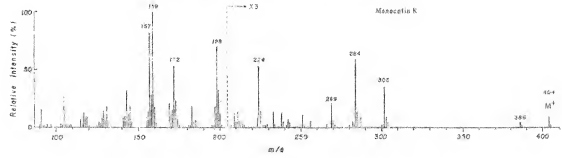
Fig. 3. ^{13}C-NMR spectrum of monacolin K.



cholesterol was determined by a conventional method using Determiner TC 5 (Kyowa Hakko Kogyo Co., Tokyo). Under these conditions, plasma cholesterol levels of the treated animals were reduced by 23.9% (mean for 5 animals, $P<0.01$).

When monacolin K was orally given to 2 rabbits (New Zealand White, 2.7~2.9 kg) twice a day (at 9 a.m and 5 p.m.) at a dose of 1 mg/kg (2 mg/kg/day) for 5 days, reductions of plasma

Fig. 4. Mass spectrum of monacolin K.



cholesterol levels obtained after 3 and 5 days were 12.3 and 21.6%, respectively, in one animal and 10.1 and 26.2% in the other.

Acknowledgements

The author wish to thank Dr. M. TADA (Tokyo Noko University) for measuring the IR, NMR and mass spectra, JEOL Ltd. (Tokyo) for measuring the ^{13}C-NMR spectra and Mrs. M. TASUKE (Tokyo Noko University) for elemental analysis. Thanks are also due to Dr. R. FUNABIKI (Tokyo Noko University) and Nihon Seibutsu Zairyo Center (Tokyo) for supporting animal experiments and Kyowa Hakko Kogyo Co. (Tokyo) for kind supply of the cholesterol-determining kit Determiner TC 5.

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(Received April 27, 1979)

References

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2) TSUJITA, Y.; M. KURODA, K. TANZAWA, N. KITANO & A. ENDO: Hypocholesterolemic effects in dogs of ML-236B, a competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Atherosclerosis 32: 307~313, 1979
3) KURODA, M.; Y. TSUJITA, K. TANZAWA & A. ENDO: Hypocholesterolemic effects in monkeys of ML-236B, a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Lipids, in press